EXHIBIT 20

Pergamon

0163-7258(94)E0024-V

Pharmac. Ther. Vol. 63, pp. 199–207, 1994
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0163-7258/94 $26.00

Associate Editor: S. PESTKA

METABOLIC SUICIDE GENES IN GENE THERAPY

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Abstract—This article reviews uses of metabolic suicide genes in gene therapy. Suicide genes encode novel nonmammalian enzymes that can convert a relatively nontoxic prodrug into a highly toxic agent. Cells genetically transduced to express such genes essentially commit metabolic suicide in the presence of the appropriate prodrug. Three metabolic suicide genes are described: herpes simplex thymidine kinase, *Escherichia coli* cytosine deaminase and varicella zoster thymidine kinase. Transfer and expression of these genes into mammalian cells is described. Preclinical models of suicide gene therapy of cancer and human immunodeficiency virus are discussed, and several clinical trials employing suicide genes are described.

Keywords—Suicide gene, gene therapy, thymidine kinase, cytosine deaminase, retrovirus, transfection, ganciclovir.

CONTENTS

1. INTRODUCTION

Suicide genes encode proteins that cause a cell to produce lethal intracellular toxins. This conditional lethality is accomplished by using nonmammalian metabolic enzymes and prodrugs that can be activated by only such enzymes. Suicide gene systems may be useful in a variety of

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Abbreviations—ara-ATP, adenine arabinoculeoside triphosphate; ara-M, 6-methoxypurine arabinoside; CD, cytosine deaminase; 5-FC, 5-fluorocytosine; 5-FU, 5-fluorouracil; HIV, human immunodeficiency virus; HSV-1, herpes simplex type-1; HSV-1-TK, herpes simplex type-1-thymidine kinase; LTR, long terminal repeat; *tat*, HIV *trans*-activator gene; VZ-TK, varicella virus-thymidine kinase.

circumstances, ranging from safety systems for gene therapies to cancer treatment and to control of human immunodeficiency virus (HIV) infection. The development of relatively efficient gene transfer systems may allow clinical application of such systems in the near future. This review will describe several suicide gene systems, discuss preclinical models and conclude with a description of several human gene therapy protocols that employ such systems.

2. METABOLIC SUICIDE GENE SYSTEMS

The basic principle underlying metabolic suicide gene systems is intracellular conversion of a relatively nontoxic prodrug to a toxic drug by an enzyme that is not normally present in the cell. Viruses, bacteria and fungi often utilize unique metabolic pathways not used by mammalian cells, and contain genes for enzymes that perform metabolic conversions that mammalian cells do not perform. Such distinctive enzymes have often been the target of drugs developed for the treatment of infections. Such agents are lethal or inhibitory for the infecting microbe, but do not harm the host cell because it lacks the enzyme system needed to activate the drug. Transfer of genes for such enzymes to mammalian cells can confer upon these altered cells the same distinctive metabolic sensitivity. If such genetically altered cells are produced in or placed in a host, treatment of the host with the prodrug will produce toxicity confined to the altered cell and/or its microenvironment without generation of significant systemic toxicity. This is so for two reasons. First, normal cells will not express the necessary activating suicide gene. Second, the active toxins generated by the suicide gene enzyme interfere with nucleic acid metabolism, and thus, only cycling cells will be susceptible to damage. Several enzyme/prodrug systems have been developed and have been successfully used *in vivo*.

2.1. HERPES THYMIDINE KINASE

Herpes simplex virus type-1 (HSV-1) is a common human pathogen. It produces a thymidine kinase (HSV-1-TK) whose substrate specificity is distinct from normal cellular thymidine kinase. This enzyme has been the target of drugs such as the guanosine analogues acyclovir and ganciclovir (Hirsch and Kaplan, 1990; Elion, 1982). Cells infected with HSV-1 contain the HSV-1-TK enzyme and will produce the monophosphate form of these drugs, while uninfected cells will not. Normal cellular guanylate kinase will then further metabolize the monophosphate to the diphosphate form (Miller and Miller, 1980). Activation is completed by other kinases (phosphoglycerate kinase, pyruvate kinase and phosphoenolpyruvate kinase), and ultimately, the highly toxic triphosphate forms of the prodrugs are generated. Production of the monophosphate is the rate-limiting step in the generation of the active form of the drugs. The triphosphate forms cause some inhibition of cellular α-DNA polymerase, and produce additional toxic effects through incorporation into DNA and chain termination (Mar *et al.*, 1985). Ganciclovir is more potent than acyclovir in this regard; it is a better substrate for HSV-1-TK (Biron *et al.*, 1985).

Cloning and transfer of the HSV-1-TK gene to normal cells confers the same selective sensitivity to these nucleic acid analogues. In the absence of the prodrug, constitutive expression of the HSV-1-TK gene is not harmful. Altered cells grow normally *in vitro* and *in vivo*. In fact, transgenic mice expressing the HSV-1-TK gene have been produced. *In vitro* cells expressing the HSV-1-TK gene are inhibited by ganciclovir in the 1–50 μM range, levels that can be achieved in patients treated with the drug.

2.2. CYTOSINE DEAMINASE

Cytosine deaminase (CD) is an enzyme found in many bacteria and fungi, but not in mammalian cells. Its normal function is to deaminate cytosine to uracil in times of nutritional stress (Danielsen *et al.*, 1992). In treatment of fungal and bacterial infections, this metabolic step has been the target of the drug 5-fluorocytosine (5-FC). Susceptible microbes deaminate 5-FC to 5-fluorourcil (5-FU), which is highly toxic. 5-FU is further metabolized to 5-fluorouridine 5′-triphosphate and 5-fluoro-2′-deoxyurdine 5′-monophosphate, which interfere with RNA and DNA synthesis, respectively. Humans and other mammals do not deaminate the 5-FC, and thus, the agent has relatively little systemic toxicity. In fact, most of the toxicity seen in humans treated with oral 5-FC

is probably due to conversion of the drug to 5-FU by bacteria living in the gut, followed by absorption of the toxic form.

The *Escherichia coli* CD gene has been modified and expressed in mammalian cells (Mullen *et al.*, 1992; Austin and Huber, 1993). In the absence of the prodrug 5-FC, modified cells grow normally. However, if 5-FC is present, the CD+ cells are inhibited. In cells containing a single copy of the CD gene under the influence of a constitutive retroviral long terminal repeat (LTR) promotor, CD+ cells can be inhibited by 5-FC in the range of 30–500 μg/ml. Such serum levels may be achievable in humans. A non-genetic approach using this system couples the CD enzyme to monoclonal antibodies, specifically targeting tumor cells (Senter, 1990; Senter *et al.*, 1991). Treatment of tumor cells *in vitro* with these enzyme–antibody conjugates and 5-FC has resulted in selective tumor cell killing.

2.3. Varicella Thymidine Kinase

Varicella virus also expresses a unique thymidine kinase (VZ-TK), whose substrate specificity differs from cellular kinases and is also distinct from HSV-1-TK. 6-Methoxypurine arabinoside (ara-M) is selectively monophosphorylated by varicella-zoster-infected cells (Averett *et al.*, 1991). This monophosphate is further metabolized by cellular enzymes (AMP deaminase, adenylosuccinate synthetase lyase, AMP kinase and nucleoside diphosphate kinase) to the highly toxic form, adenine arabinonucleoside triphosphate (ara-ATP). When mammalian cells are transduced with the VZ-TK gene, they are significantly inhibited by ara-M at concentrations of 1–100 μM (Huber *et al.*, 1991). Normal cells regularly withstand >1500 μM ara-M. Sensitivity to the prodrug, ara-M, is directly proportional to the VZ-TK activity in the cell.

3. PRECLINICAL MODELS EMPLOYING SUICIDE GENES/GENE DELIVERY

3.1. Transgenic Mice Expressing HSV-1-TK

While useful *in vitro*, e.g. in studies of homologous recombination (Mansour *et al.*, 1988), additional utility of metabolic suicide genes comes with expression *in vivo*. One of the earliest applications of a suicide gene came in studies of lymphoid reconstitution in transgenic mice engineered to express the HSV-1-TK enzyme (Borrelli *et al.*, 1988). An expression construct placing the HSV-1-TK gene under the influence of the immunoglobulin heavy chain enhancer and the light chain promoter was transferred into fertilized mouse cells. A strain expressing HSV-1-TK was eventually derived from these cells. The enzyme was expressed only in lymphoid organs because of the tissue specificity of the promoter and enhancer. Without prodrug treatment, the transgenic animals were similar in size and organ weight to normal mice. However, a 7-day infusion of acyclovir or ganciclovir led to an 80–90% reduction in the cellularity of thymus, spleen, bone marrow and lymph nodes, with little change in other organs. Cellular depletion correlated with HSV-1-TK enzyme activity. These elegant studies demonstrated the feasibility of suicide gene expression *in vivo* and the potential utility of tissue specific promoters in regulating their expression.

3.2. Elimination of Tumors with Suicide Genes

Cancer chemotherapists seek agents that selectively kill tumor cells, but spare nonmalignant tissues. In general, this search has been unsuccessful because tumor cells use essentially the same metabolic pathways as normal cycling cells. The observation of selective killing of suicide gene-modified cells by the appropriate prodrugs suggests that if tumors can be manipulated to express these suicide genes, it is theoretically possible that systemic treatment of the host with the prodrug may have a significant impact on the tumor.

This possibility has been tested by a number of investigators working with the HSV-1-TK gene in a variety of tumor models. In such studies, tumor cells have been manipulated *in vitro* to express the HSV-1-TK gene and then reinjected into syngeneic hosts. Animals then received parenteral treatment with acyclovir or ganciclovir, and tumor growth was monitored. HSV-1-TK positive clones of a murine sarcoma line transfected by calcium phosphate precipitation were shown to be

sensitive *in vitro* to 0.1–1 μM ganciclovir. When these tumor lines were injected into animals receiving a 5-day course of ganciclovir (twice daily at a dose of 150 mg/kg/dose) begun about 1 week after tumor injection, treated mice consistently failed to grow tumors, while untreated mice exhibited progressive tumor growth. HSV-1-TK-negative tumors were unaffected by prodrug treatment (Moolten, 1986). Similar results were seen in cloned sarcoma lines transduced *in vitro* with retroviral vectors, although about one-third of the mice exhibited tumor recurrences once drug therapy was discontinued (Moolten and Wells, 1990; Moolten *et al.*, 1992). The majority of recurrent tumors no longer expressed the HSV-1-TK gene. Murine adenocarcinoma clones transduced with an HSV-1-TK retroviral vector regressed in animals receiving oral ganciclovir 75–100 mg/kg/day for 14 days. Even animals whose drug treatment began more than 1 month after tumor inoculation, and whose tumors were greater than 4 cm in diameter, regressed (Plautz *et al.*, 1991). Glioma cells have also been shown to be susceptible to HSV-1-TK/ganciclovir-mediated killing (Ezzeddine *et al.*, 1991). Cloned HSV-1-TK-positive rat C5 glioma cells were sensitive to ganciclovir 2–5 μM *in vitro*. In xenografts in nude mice, these gliomas were suppressed by parenteral treatment with ganciclovir 50 mg/kg/dose twice daily for 12 days. A variety of other tumor types have been shown to be susceptible to HSV-1-TK/ganciclovir-mediated killing (Golumbek *et al.*, 1991; Hasegawa *et al.*, 1993; Panis *et al.*, 1992).

While the HSV-1-TK/ganciclovir system has been active *in vivo* for a variety of tumors, there is evidence that in tumors derived from hematopoietic tissues, it may be less effective. Murine lymphomas transduced with retroviral vectors were shown to be sensitive *in vitro* to ganciclovir. However, treatment *in vivo* failed to eliminate tumors (Moolten and Wells, 1990). Tumor cells emerging after ganciclovir treatment exhibited dramatically reduced HSV-1-TK activity. Human leukemia cell lines were shown to express the HSV-1-TK gene after retroviral gene transduction, and were sensitive to ganciclovir *in vitro* at 1–2.5 μM (Abe *et al.*, 1993). However, when nude mice were inoculated with one of these human leukemia lines, ganciclovir treatment (40 mg/kg/day for 15 days) arrested tumor growth, but did not eliminate the tumors. After drug treatment was discontinued, the tumors resumed growing. At this point, it is not clear if this is a generalizable phenomenon or represents the activity of the promoter and vector. In these studies the gene was transferred by a retroviral vector. However, HSV-1-TK-positive lymphomas have been shown to be sensitive to HSV-1-TK/ganciclovir-mediated killing in HSV-1-TK-positive transgenic mice (Moolten *et al.*, 1990). Eleven of 12 primary lymphomas induced by Abelson leukemia virus in transgenic mice expressing the HSV-1-TK gene under the influence of a lymphoid specific promoter (discussed in Section 3.1) regressed completely in mice treated with ganciclovir (140 mg/kg/dose twice daily for 5 doses). Treatment was started when palpable tumor was detected. Six of the 11 responders recurred within 100 days of treatment and seemed to have lost HSV-1-TK activity.

In vivo expression of other suicide gene systems is being explored. To date, evidence seems to indicate that CD can also function efficiently in some circumstances to allow elimination of CD positive tumors with 5-FC.

3.2.1. *Delivery of Suicide Genes* In Vivo

The demonstration that tumors manipulated *ex vivo* and reinjected into a host can be eliminated by the appropriate prodrug is encouraging, but, in itself, is not of obvious clinical utility. The important therapeutic question is whether tumor in a host can be rendered sensitive *in situ* to the prodrug. Thus, gene delivery *in vivo* becomes a very important issue in metabolic suicide gene schemes.

Methods effective *in vitro* for transferring genes, such as calcium phosphate precipitation, electroporation and exposure to viral vectors, are not efficient *in vivo*. Direct injection of DNA, DNA–liposome complexes, DNA–metal complexes or retroviral vectors have all been shown to transfer DNA into cells, but at efficiences of less than 1% of target cells. To increase exposure of tumor cells to vector, alternate methods have tried to deposit within tumor cells that can continuously manufacture and secrete retroviral vectors.

"Producer lines" are cell lines (usually fibroblast-derived) that have been genetically engineered to produce replication-defective retroviral vectors (Miller, 1990). The vectors, themselves, are defective viruses that lack genes (*gag*, *pol* and *env*) needed to manufacture viral structural and

regulatory proteins (Miller and Rosman, 1989). The absence of these genes prevents the vector from replicating in target cells and propagating the infection. These important viral genes have been replaced by the sequences one wishes to transfer to target cells. The producer line makes the needed viral proteins, complementing the genetic defect in the vector. Thus, only in these cells can vectors reproduce, while in other target cells, the vectors cannot reproduce.

On approach to *in vivo* tumor transduction is the injection of retroviral vector-producer cells into a tumor mass. The hope is that the producer cells within the tumor mass would continuously secrete vector for several days at least, increasing the exposure of the tumor cells to vector. This approach was validated by experiments in which a producer line making a β-galactosidase vector was inoculated into gliomas growing in rat brain (Short *et al.*, 1990). Histochemical evidence of β-galactosidase gene transfer was seen in 1–10% of the glioma cells, while normal brain was not transduced. (The tumor specificity of the gene transduction is attributable to the fact that retrovirus vectors integrate into replicating cells, while noncycling cells are resistant.) Direct injection of vector preparations without the producer lines produced very little tumor transduction. The HSV-1-TK gene has been transferred with a similar technique, and efficiencies of 10–70% have been claimed (Culver *et al.*, 1992; Ram *et al.*, 1993). Small intracerebral gliomas in rats were stereotactically injected with HSV-1-TK vector-producer cells (10- to 100-fold more producer cells were injected compared with the number of glioma cells inoculated 7 days earlier). Then, 5–7 days later the rats received a course of ganciclovir. Under these conditions, about 75% of tumors regressed, as assessed by autopsy roughly 1 month after tumor inoculation. Long-term survival data was not reported, and thus, tumor recurrence rates after such treatment cannot be estimated.

An alternate approach introduced live, wild-type, replication-competent retrovirus along with HSV-1-TK vector (Takamiya *et al.*, 1992, 1993). The presence of wild-type "helper virus" has the effect of propagating infection in the tumor itself. Tumor cells infected with wild-type virus and HSV-1-TK vector can produce both more wild-type virus and HSV-1-TK vector, while HSV-1-TK vector targeted cells not infected by helper virus cannot produce more vector. In nude mice injected with a mixture of both tumor cells lacking the HSV-1-TK gene and tumor cells infected with both wild-type virus and HSV-1-TK vector, regression of the mixed tumor was seen after treatment with ganciclovir. This observation would be consistent with the spread of the HSV-1-TK vector throughout the tumor. However, the contribution of metabolic "bystander killing" cannot be excluded.

3.2.2. *"Bystander Killing" of Non-Transduced Cells*

A feature shared by these suicide genes is the selective activation of prodrug by only those cells transduced with the gene. Thus, a population of suicide gene-modified cells will die in the presence of the prodrug, while a population of unmodified wild-type cells will continue to grow and metabolize normally. An important question arises: what happens in mixed populations? While only the genetically modified cells will activate the prodrug, it is possible that wild-type cells in the immediate vicinity of the modified cell may be affected by the toxic metabolite generated by the suicide gene harboring cell.

"Bystander killing" of unmodified cells has been observed in the HSV-1-TK system. The results of the studies discussed in Section 3.2.1 in which gene transfer *in vivo* was attempted point to the phenomenon. Gene transfer efficiency was reported as 10–70%; yet, in many animals, complete tumor regression was observed. Presumably, 30–90% of the cells in these regressing tumors did not harbor the suicide gene and yet were inhibited when their HSV-1-TK-positive neighbors were treated with ganciclovir. Inhibition of unmodified tumor cells by HSV-1-TK-positive tumor cells has also been observed in mixed tumors in which no vector-producing cells have been inoculated (Culver *et al.*, 1992). In a fibrosarcoma model, tumors in which only 50% of the cells were HSV-1-TK-positive, regression was as complete as in 100% HSV-1-TK-positive tumors. Even in mixed tumors in which only 10% of the cells were HSV-1-TK-positive, over one-half of tumors regressed with ganciclovir treatment. This apparent "bystander killing" of unmodified tumor cells was tentatively explored *in vitro* in the earliest reports of the HSV-1-TK system (Moolten, 1986). There it was observed that when mixtures of HSV-1-TK-positive and -negative cells were plated at very low density *in vitro*, ganciclovir treatment eliminated only the HSV-1-TK-positive cells.

When the same cell mixture was plated at high density, nearly all cells, both HSV-1-TK-positive and -negative, were killed. It was speculated that some type of metabolic cooperation between adjacent cells allowed transport of activated drug. This phenomenon is under investigation in a number of laboratories. Preliminary evidence has suggested several possibilities, ranging from phagocytosis of toxin-laden apoptotic vesicles produced by suicide gene expressing dying cells to intracellular transport of the phosphorylated drug through intercellular junctions.

While the mechanism of "bystander killing" in neighboring tumor cells has not been fully elucidated, it is clear that normal tissues in the host are not damaged by the suicide gene system. While toxin concentrations may be high in the microenvironment, they seem to be diluted effectively, such that systemic toxic concentrations are not reached. The many studies of animals bearing HSV-1-TK-positive tumors are in agreement that mice do not suffer systemic toxicity when treated with ganciclovir.

While systemic toxicity has not been observed, one can still ask if normal tissues in the microenvironment of the HSV-1-TK-positive tumor would be damaged. This has not been observed to date, either with inoculation of HSV-1-TK-positive cells alone or with addition of vector-producing cells. Non replicating normal tissues are resistant to both retroviral transduction and the antimetabolites generated by the suicide gene.

"Bystander killing" has been most extensively studied in the HSV-1-TK system. There are unpublished preliminary reports that some bystander killing occurs in the cytosine deaminase system.

3.3. CONTROL OF HIV INFECTION

3.3.1. *Tissue Specificity of Suicide Gene Expression*

A further measure of selective toxicity can be conferred by coupling suicide genes to tissue-specific or -inducible promoters. Placement of the VZ-TK gene under α-fetoprotein regulatory elements or the albumin promoter led to selective expression in hepatic tumor cells (Huber *et al.*, 1991). The selective ablation of lymphoid tissues in the HSV-1-TK transgenic mouse, in which the gene was under the influence of lymphoid-specific regulatory elements, is another piece of elegant evidence attesting to the potential specificity afforded by tissue specific promoters.

3.3.2. *HIV-Inducible Suicide Genes*

Another form of tissue-specific expression was exploited in a potential anti-HIV therapy based on a suicide gene (Caruso and Klatzmann, 1992). The HSV-1-TK gene was cloned into an expression vector under the influence of the HIV LTR, and the resulting plasmid was transferred into a $CD4^+$ lymphoid cell line (HUT-78) by electroporation. Clones expressing the gene were then analyzed. The HIV LTR is an inducible promoter whose activity is substantially increased in the presence of TAT, one of the HIV-regulatory proteins that appears relatively early in HIV replication. In the absence of TAT, the HIV LTR promotes very little gene expression. Thus, in uninfected cells, there should be little HSV-1-TK, while in cells infected with HIV, the promoter will be induced by the TAT protein and substantial amounts of HSV-1-TK enzyme should be produced. If acyclovir or ganciclovir are present, then HIV-infected cells should commit metabolic suicide early in the HIV replicative cycle, and spread of the infection should be controlled. *In vitro*, there was evidence that the presence of 10 μM acyclovir abolished the replication of HIV in the HSV-1-TK-positive HUT-78 clones, and that some cultures were effectively sterilized. Testing of such schemes *in vivo* is underway.

3.4. LIMITATIONS TO SUICIDE GENE APPLICATIONS

While these observations in animal models are encouraging, enthusiasm for possible use of suicide genes in human therapies should be tempered by consideration of their limitations. First, there remain formidable technical obstacles to efficient delivery of genes to tissues *in vivo*. Even

under nearly optimal circumstances in preclinical models, the majority of target cells are not successfully transduced. Second, delivery of a vector to a cell still does not guarantee efficient expression of its genes. Populations of cells transduced with retroviral vectors will include some cells that exhibit high levels of gene expression and many others that contain little gene product. The potency of the suicide gene systems generally is related directly to the amount of enzyme activity produced. Third, clinical tumors generally have a lower growth fraction or mitotic index than experimental murine tumors. Tumor cells not actively cycling at the time of gene transfer or metabolic activation may be resistant to both genetic transduction and the toxic effects of the activated drugs. Fourth, tumor cells are inherently genetically unstable. It is likely that within a large population of suicide gene-transduced tumor cells (even assuming initially efficient gene transfer), variants will be generated that cease to express the suicide gene adequately. Such cells could easily escape and produce recurrent tumor.

4. CLINICAL TRIALS EMPLOYING SUICIDE GENE SYSTEMS

Despite the aforementioned limitations, several schemes for human gene therapy employing the HSV-1-TK suicide vector have been proposed. Patients have been enrolled in several of these trials, but assessments of the activity of the systems will not be available before mid-1994. The basic principles of these proposed clinical trials will be summarized below.

4.1. HSV-1-TK as a Safety System in Adoptive Immunotherapy

One proposed use of a suicide gene in a clinical trial employs HSV-1-TK as a safety system rather than as a therapeutic tool (Riddell *et al.*, 1992). The trial is an adoptive immunotherapy scheme for HIV-related malignancies in which large numbers of autologous cytolytic lymphocytes will be infused into HIV-positive patients undergoing allogeneic bone marrow transplantation for non-Hodgkin's lymphoma. Autologous cytolytic T-cells specific for HIV antigens will be transduced with a vector containing an HSV-1-TK gene. This cell population will then be expanded *in vitro* and infused into the patient following the transplant. A goal of this procedure is T-cell-mediated eradication of residual HIV virus and virus-infected cells in the patient. Since the T-cells will have been transduced with HSV-1-TK, if any untoward effects develop in recipients of the cytolytic T-cells, it is hoped that the patient can be infused with ganciclovir and the offending HSV-1-TK-positive T-cells will be eliminated.

4.2. HSV-1-TK Gene Transfer into Intracerebral Tumors

Another group of clinical trials attempts to use HSV-1-TK to treat intracerebral tumors, focusing on *in vivo* gene transduction and relying heavily upon "bystander killing" of non transduced tumor cells (Oldfield *et al.*, 1993). In this scheme, HSV-1-TK retroviral-producer lines will be injected into intracerebral tumors (either primary brain tumors or cerebral metastases from other tumors) and 7 days later patients will receive a 14-day course of ganciclovir (5 mg/kg/dose twice a day). It is hoped that these human tumors will regress like the rat glioma tumors described in Section 3.2.1. Significant differences between the experimental model and the human tumors may limit the success of these initial human experiments. The human tumors will be larger, will significantly outnumber the injected producer cells (the converse of the rat model) and they will have a lower growth fraction. Initial reports from one of these trials indicate that some tumors demonstrate some response, but that local recurrences occur regularly.

4.3. Allogeneic HSV-1-TK Tumor Cells for Treatment of Ovarian Carcinomatosis

Another approach also relies heavily on "bystander killing". The proponents of this trial reported in their application to the Recombinant DNA Advisory Committee that intraperitoneal injection of thymidine kinase-modified tumor cells and systemic treatment with ganciclovir of mice with peritoneal carcinomatosis prolonged survival (Recombinant DNA Advisory Committee,

1991). They speculated that HSV-1-TK-positive cells metabolized ganciclovir to its toxic form, died, and unmodified tumor cells took up toxin-laden vesicles of dead cells by endocytosis. Immunologic effects were also implicated, as the strategy is more effective in immunocompetent mice. Based on these observations, a trial in ovarian carcinomatosis is being planned. A human ovarian carcinoma line will be transduced *in vitro* with an HSV-1-TK-retroviral vector. Patients with ovarian carcinomatosis will receive intraperitoneal injections of these allogeneic HSV-1-TK-positive cells followed by systemic treatment with ganciclovir. It is hoped that only tumor cells, but not normal tissues, will take up the products of the HSV-1-TK-positive cells.

5. CONCLUSION

Gene transfer technology has allowed expression of novel metabolic enzymes in mammalian cells, making them capable of metabolically activating potentially active prodrugs. These metabolic suicide genes have been of significant use in basic science, having been employed in studies of homologous recombination and lymphoid development. Their ability to confer novel chemosensitivity upon tumor cells and HIV-infected lymphoid cells has prompted exploration of their potential utility in the treatment of malignancies and HIV infection. While it remains to be seen whether they will have any real impact clinically, they indicate the potential power of modern molecular biology, and may presage other clinical interventions based on genetic technologies.

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